EGL Genetics | Eurofins Clinical Diagnostics

EGL Genetics Laboratory Classification Definitions

EGL Genetics Molecular Laboratory classifies sequencing variants following the guidelines of the ACMG Laboratory Practice Committee Working Group (Richards et al., 2015. Genet Med. 2015 May;17(5):405-23; Richards et al 2008. Gen in Med. 10: 294-300) and Ogino et al 2007. Journal of Molecular Diagnostics, Vol 9, No 1:1-6. These guidelines represent a basic framework for interpretation of sequence variants. Each variant is individually assessed in the context of the variant, gene, associated disease and patient phenotype.

Sequence variants are classified in one of six categories. Below is a summary of the guidelines used by our laboratory (as of 2015). Please note, the following applies to variants in genes associated with disease (GADs). Any variant in a gene of unknown significance (GOUS) will be classified as a variant of uncertain significance (VOUS).

Genes known to be associated with specific phenotypes (single and targeted panel gene sequencing)

1. **Pathogenic**
    a. Variants predicted to result in the loss of protein function in a gene for which this is a known mechanism of disease (may or may not have been previously reported in patients with disease)
        1. frameshift (an insertion or deletion that is not a multiple of 3 nucleotides)
        2. nonsense (introduction of a premature stop codon)
        3. splice junction (at positions +1,+2, -1 and -2 in an intron unless there is data from the literature or databases to suggest the change is not pathogenic)
        4. change in an initiation codon if an initiation codon change has been shown to be a disease mechanism
        5. change in the termination codon if previously demonstrated to be associated with disease
    b. Variants predicted to result in an amino acid replacement (missense) with one of the following conditions met:
        1. variant demonstrated to result in reduced protein function (loss of function), or aberrant protein function (gain of function) in an appropriate functional assay
        2. common disease causing pathogenic variant in a specific population based on evidence in the literature
        3. variant reported in multiple affected individuals and demonstrated to segregate with disease in multiple families (Note: the number of families depends on the gene and the disease with which it is associated. The mode of inheritance of the disease is also considered; e.g. autosomal recessive vs dominant.)
    c. Variants demonstrated to result in aberrant splicing in an appropriate functional assay (e.g. intronic or silent)

2. **Likely Pathogenic**
    a. Recessive conditions: (all the following conditions must be met)
        1. diagnosis has been confirmed by biochemical testing or patient phenotype is specific for disease
        2. variant located on opposite chromosome from a known disease causing pathogenic or likely pathogenic variant
        3. variant occurs at an evolutionarily conserved nucleotide and/or amino acid
        4. variant not present in dbSNP, 1000 Genomes Project, EVS (Exome Variant Server), ExAC, or other publically available database at a frequency consistent with being a benign variant
    b. Dominant conditions: (all of the following conditions must be met)
        1. variant segregates with phenotype in the family being tested (Note: reduced penetrance is considered in the evaluation.) **or**
        2. testing parental samples demonstrates that the variant occurred *de novo*
        3. variant not present in dbSNP, 1000 Genomes Project, EVS, ExAC, or other publically available database at a frequency consistent with being a benign variant

3. **Benign variant** (one of the following conditions must be met)*
    a. Variant reported in dbSNP, 1000 Genomes Project, EVS, ExAC, locus specific databases or EGL database at a population frequency higher than expected given the prevalence of the disease and mode of inheritance.
    b. Variant reported in a control population at a frequency inconsistent with being causative of disease
    c. Other evidence from published literature that indicates the variant has no effect on function

4. **Likely benign variant (**one of the following conditions must be met)
    a. Variant found heterozygous (for dominant) or homozygous (for recessive) in multiple unaffected individuals at an allele frequency inconsistent with clinical significance based on mode of inheritance and severity of disorder
    b. Variant found in *cis* with a pathogenic variant in multiple unrelated individuals
    c. Variant found in an unaffected family member (for dominant)

*Benign and likely variants are interpreted as described above and not reported in clinical reports. A list of these variants is available upon request.

5. **Variant of uncertain clinical significance (VOUS)** (if unable to classify the variant in one of the four categories above, it will be classified as a VOUS)
    a. Variant not reported in HGMD, locus specific databases, published literature, dbSNP, 1000 Genomes Project, EVS, or ExAC
    b. Variant reported in dbSNP,1000 Genomes Project, EVS, or ExAC, but at an allele frequency insufficient to rule out clinical significance based on mode of inheritance and severity of disorder
    c. Variant reported in a single individual with insufficient segregation and/or functional data
    d. Variant reported in a single individual with inadequate clinical information
    e. Evidence from multiple sources is conflicting (i.e. evidence for both a pathogenic and benign classification exists)
6. **Other reportable** – variant is clinically benign (not associated with disease) but is reported when observed (e.g. pseudodeficiency alleles)

Exome: In clinical whole exome sequencing, for a variant to be classified as a pathogenic variant it must be located in a gene associated with disease (GADs). Additionally, the phenotype of the patient being tested must be consistent with that disease. Other sequence variants are reported according to the preferences selected by the patient on the consent form. Variants in genes of unknown significance (GOUSs) will be classified as variants of uncertain significance. EGL will attempt to investigate the possibility of a new gene: disease association if a strong suspicion of the GOUS being associated with disease arises.